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# Susceptibility of European bread and durum wheat cultivars to *Tilletia indica*

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Representative European wheat cultivars were tested under quarantine containment for their susceptibility to *Tilletia indica,* the cause of Karnal bunt of wheat. Fifteen winter and 15 spring wheat (*Triticum aestivum*) and 11 durum wheat (*Triticum durum*) cultivars were inoculated by boot injection just prior to ear emergence to test their physiological susceptibility. Selected cultivars were then re-tested by spray inoculation after ear emergence to determine their morphological susceptibility, which is a better predictor of field susceptibility. At maturity, the ears and seeds were assessed for incidence and severity of disease. For the physiological susceptibility tests, 13/15 winter wheat cultivars were infected and the percentage of infected seeds ranged from 1 to 32%. For spring cultivars, 13/15 cultivars were infected and the percentage of infected seeds ranged from 1 to 48%. For the durum cultivars, 9/11 were infected and the percentage of infected seeds ranged from 2 to 95%. Across all cultivars, 35/41 were infected. Based on historical Karnal bunt susceptibility categories using coefficients of infection, one cultivar was classed as highly susceptible, three as susceptible, 11 as moderately susceptible, 20 as resistant and only six as highly resistant. The spray-inoculation morphological susceptibility tests broadly confirmed the physiological susceptibility results, although lower levels of infection were observed. Overall, the range of susceptibility was similar to that found in cultivars grown in Karnal bunt affected countries. The results demonstrate that European wheat cultivars are susceptible to *T. indica* and thus could potentially support the establishment of *T. indica* if introduced into Europe.

*Keywords*: Karnal bunt, Pest Risk Analysis, physiological and morphological susceptibility, *Triticum aestivum*, *Triticum durum*

## Introduction

Karnal bunt of wheat, caused by the fungus *Tilletia indica*, is a floret-infecting smut pathogen of wheat (*Triticum* spp.) and triticale (× *Triticosecale*). In broad terms, the fungus infects the glumes via the rachis from the awns emerging stage, through heading, to late flowering. Seeds are infected through the germinal end of the grain and the fungus develops within the pericarp where it produces a powdery, brownish-black mass of teliospores (Goates, 1988). The spore masses produce a foetid, decaying fish-like smell (trimethylamine). Naturally infected seeds are usually only partially colonized, showing various degrees of infection. Point infections are most common, but infection may also spread down the adaxial groove and, in severe cases, the whole seed may appear bunted. Since infection occurs late in the phenological development of the host, the disease is more difficult to control than systemic smuts (e.g. *Tilletia tritici*), which can usually be controlled effectively using fungicide seed treatments (Hoffmann & Waldher, 1981).

Karnal bunt was first formally recorded in 1930 in the north Indian city of Karnal (Mitra, 1931). The pathogen has since been recorded in other Asian countries, including Afghanistan, Iraq, Nepal and Pakistan (Warham, 1986), and Iran (Torabi *et al*., 1996). It has also been introduced to several non-Asian countries, including Mexico (Durán, 1972), the southern part of the Rio Grande do Sul in Brazil ((Da Luz *et al*., 1993), the USA (Ykema *et al*., 1996) and most recently in the northern Cape Province of South Africa (Crous *et al*., 2001).

Its introduction and establishment in south-west areas (Arizona, California and Texas) of the USA (Rush *et al*., 2005) created another significant new trade pathway for entry into Europe. As a consequence, a UK Pest Risk Analysis (PRA) (Sansford, 1996, 1998) was produced that

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assessed the risk of establishment and economic damage to the UK and wider European Union (EU). As a result, *T. indica* was added as an I/AI quarantine pest to the EC Plant Health Directive 77/93/EEC (now 2000/29/EC) in 1997. The EC Directive applies certain quarantine requirements to seed and grain of *Triticum* and × *Triticosecale* from countries where *T. indica* is known to occur. Since 1996, interceptions of *T. indica* on wheat grain for consumption/processing imported to Europe have been reported by four countries: by Poland in grain from India in 1996; by Italy on durum wheat from Mexico in 1998 and 2006; by the UK in five consignments of grain from India from 2003 onwards; and possibly by Greece on US grain in 1996 (Inman *et al*., 2008; I. Vloutoglou, Benaki Phytopathological Institute, Greece, personal communication).

The UK PRA predicted a risk of entry based principally on trade pathways, a risk of establishment in Europe and the potential for economic damage through quality losses (downgrading of milling wheat to feed wheat) as well as losses in export markets. Conclusions were formulated from the existing biological information in the available literature and a published disease model (Jhorar *et al*., 1992). As the fungus is still not present in Europe, data on the susceptibility of European wheat cultivars to *T. indica* were not available, nor was other information on the behaviour of the pathogen in relation to European climatic conditions, such as teliospore survival (Inman *et al*., 2008), or dormancy and germination (Peterson *et al*., 2006). An EU-funded research project ('Karnal bunt risks', Project No. QLK5-1999-01554) aimed to produce a more accurate assessment of risk to the EU based upon experimentation and specific scientific objectives. One of these objectives was to determine the susceptibility of European bread (*Triticum aestivum*) and durum (*Triticum durum*, syn.: *T. turgidum* var. *durum*) wheat cultivars to *T. indica*. This objective arose because cultivar susceptibility would affect the likelihood of establishment of *T. indica* and influence subsequent economic losses. Further, the difficulty and cost of control would depend on whether resistant cultivars could be easily developed. The reactions of 41 European wheat cultivars are reported here and these results have contributed to the new EU PRA for *T. indica* (Sansford *et al*., 2006).

## Materials and methods

All work with *T. indica* was performed under quarantine containment at the Central Science Laboratory (CSL), UK and at CRA-Centro di Ricerca per la Patologia Vegetale (CRA-PAV), Italy, with the respective Government permits or licences. Detailed methods are reported elsewhere (Porta-Puglia *et al*., 2002; Riccioni *et al*., 2004), but are given in brief below.

### Selection of European wheat cultivars

Seeds of the most widely grown wheat cultivars (winter, spring and durum), or emerging cultivars, were collected from wheat growing areas of northern and southern Europe, and a few from central and eastern Europe. Information on their provenance and end-use (e.g. bread wheat, biscuit wheat, feed wheat) can be found in Porta-Puglia *et al*. (2002). The highly susceptible Indian spring wheat cultivar WL-711 was collected for use as a comparative control.

The universally accepted standard method to test the resistance/susceptibility of wheat germplasm is boot inoculation using $1 \times 10^4$ allantoid sporidia $mL^{-1}$ injected directly into the boot cavity (Rajaram & Fuentes-Dávila, 1998) at growth stage (GS) 45 (boot swollen) (Tottman & Broad, 1987). This is generally considered to give the most reliable indication of physiological susceptibility to infection (Aujla *et al*., 1990). It is the primary method for screening cultivars for resistance under field conditions in countries where Karnal bunt occurs, such as India and Mexico. Because inoculum is placed directly inside the boot cavity, results are not significantly affected by the absence of environmental conditions normally required for natural infection to occur (GL Peterson, unpublished data). However, it is a severe inoculation method and whilst it produces high levels of infection it does not screen for morphological (field) resistance. Although more natural inoculation methods, e.g. spray-inoculation methods applied during heading or flowering, generally give much lower and less reliable levels of infection, they may more accurately represent field susceptibility because they include elements of both physiological and morphological resistance (Royer & Rytter, 1985; Warham, 1988, 1990).

For this reason, spring, winter and durum wheat cultivars, deemed to represent the distribution and variability of cultivars in the most important wheat growing areas in Europe, were chosen and initially tested for their physiological susceptibility to *T. indica*. Then, based on the results, a sub-set of each wheat type was selected and tested using spray inoculation to determine morphological susceptibility.

*Physiological susceptibility*

Fifteen European winter wheat cultivars were tested using a boot injection method at CSL, UK (Table 1A); 15 spring (Table 1B) and 11 durum wheat cultivars (Table 1C) were tested at CRA-PAV, Italy. The highly susceptible Indian spring wheat cultivar WL-711 was used as a comparative control at both sites.

*Morphological susceptibility*

Winter, spring and durum wheat cultivars were chosen with a range of physiological susceptibilities (high, medium and low; Table 2) based on the boot injection studies, and tested using a spray-inoculation method. Ten winter wheat cultivars were tested at CSL, UK, and seven spring and eight durum cultivars were tested at CRA-PAV, Italy. Two experiments were done with both the spring and the durum cultivars, since the plants of some cultivars in the first experiments did not produce good seed; data from both these morphological susceptibility experiments were combined. The Indian susceptible spring cultivar WL-711 was also chosen as a comparative control.

Table 1 Physiological susceptibility of European winter (A), spring (B) and durum (C) wheat cultivars to *Tilletia indica*: incidence (ears and seeds infected), severity (seeds per infection category), coefficient of infection (CI) and categorization of susceptibility for each cultivar compared to a highly susceptible Indian spring wheat cultivar (WL-711), following boot injection at growth stage 45

| Cultivar[a,b] | Ears infected/ total number | Seeds infected/total number (% infection) | Mean % seeds infected/infected ear | Distribution of infected seeds (%) by infection category (1–4) | | | | CI[d] | Susceptibility category[e] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | | |
| A – winter wheat cultivars | | | | | | | | | |
| Aztec[c] (B) | 6/10 | 15/251/(6) | 8·7 | 100·0 | 0·0 | 0·0 | 0·0 | 1·5 | R |
| Centauro[c] (A) | 8/10 | 63/195/(32) | 40·6 | 55·6 | 30·2 | 7·9 | 6·4 | 13·3 | S |
| Charger (A) | 7/10 | 21/199/(11) | 26·8 | 95·2 | 4·8 | 0·0 | 0·0 | 2·8 | R |
| Claire (A) | 2/10 | 6/308/(2) | 12·0 | 100·0 | 0·0 | 0·0 | 0·0 | 0·5 | R |
| Drifter (A) | 3/10 | 4/294/(1) | 5·4 | 75·0 | 25·0 | 0·0 | 0·0 | 0·4 | R |
| Equinox (A) | 4/10 | 9/205/(4) | 13·1 | 100·0 | 0·0 | 0·0 | 0·0 | 1·1 | R |
| Flair (A) | 1/10 | 2/214/(1) | 10·0 | 50·0 | 50·0 | 0·0 | 0·0 | 0·4 | R |
| Isengrain[c] (B) | 1/8 | 1/98/(1) | 14·3 | 100·0 | 0·0 | 0·0 | 0·0 | 0·3 | R |
| Kosack (B + C) | 11/18 | 57/265/(21) | 42·7 | 24·6 | 45·6 | 17·6 | 12·3 | 11·7 | S |
| Kris (B) | 0/8 | 0/95/(0) | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 | HR |
| Malacca (A) | 0/10 | 0/298/(0) | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 | HR |
| Mikon (A) | 4/10 | 28/343/(8) | 21·4 | 50·0 | 17·9 | 14·3 | 17·9 | 4·1 | R |
| Mjølner (B + C) | 4/7 | 18/81/ (22) | 46·8 | 77·8 | 16·7 | 5·6 | 0·0 | 7·1 | MS |
| Serio[c] (A) | 8/10 | 81/287/(28) | 35·4 | 71·6 | 21·0 | 7·4 | 0·0 | 9·6 | MS |
| Soisson[c] (B) | 1/9 | 3/162/(2) | 10·0 | 66·7 | 33·3 | 0·0 | 0·0 | 0·5 | R |
| WL-711 (A + B + C) (control) | 25/29 | 247/494/(50) | 61·3 | 52·6 | 25·1 | 11·7 | 10·5 | 22·5 | HS |
| WL-711 (A) (water control) | 0/10 | 0/167/(0) | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 | – |
| B – spring wheat cultivars | | | | | | | | | |
| Anza (D) | 8/10 | 49/137/(36) | 63·0 | 57·1 | 20·4 | 18·4 | 4·1 | 14·4 | S |
| Axona (H) | 0/5 | 0/89/(0) | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 | HR |
| Bolero (G) | 4/10 | 30/202/(15) | 51·5 | 76·6 | 6·7 | 10·0 | 6·7 | 4·9 | R |
| Cartaya (F) | 2/7 | 6/57/(10) | 53·5 | 16·7 | 83·3 | 0·0 | 0·0 | 4·8 | R |
| Cézanne (I) | 2/10 | 21/187/(11) | 40·9 | 61·9 | 23·8 | 14·3 | 0·0 | 3·0 | R |
| Chablis (G) | 5/9 | 20/131/(15) | 40·6 | 35·0 | 20·0 | 15·0 | 30·0 | 8·7 | MS |
| Colfiorito (D) | 4/6 | 3/35/(9) | 93·0 | 0·0 | 0·0 | 0·0 | 100·0 | 8·6 | MS |
| Imp (F) | 1/10 | 3/214/(1) | 100·0 | 33·3 | 33·3 | 0·0 | 33·3 | 0·8 | R |
| Naxos (G) | 3/10 | 26/214/(12) | 43·3 | 65·4 | 15·4 | 7·7 | 11·5 | 4·5 | R |
| Pandas (D) | 8/10 | 62/130/(48) | 58·0 | 79·1 | 16·1 | 4·8 | 0·0 | 9·5 | MS |
| Quattro (I) | 0/4 | 0/11/(0) | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 | HR |
| Sagittario (F) | 4/10 | 24/98/(24) | 68·0 | 75·0 | 16·6 | 4·2 | 4·2 | 6·6 | MS |
| Sandra (H) | 3/10 | 16/156/(10) | 36·0 | 43·7 | 31·3 | 18·7 | 6·3 | 4·9 | R |
| Saxana (G + H) | 2/20 | 3/374/(1) | 16·1 | 66·7 | 33·3 | 0·0 | 0·0 | 0·3 | R |
| Thasos (G + I) | 9/13 | 24/161/(15) | 36·7 | 54·1 | 0·0 | 4·2 | 41·7 | 8·1 | MS |
| C – durum wheat cultivars | | | | | | | | | |
| Anton (F) | 2/8 | 5/64/(8) | 68·0 | 40·0 | 20·0 | 0·0 | 40·0 | 4·4 | R |
| Betadur (I) | 0/1 | 0/1/(0) | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 | HR |
| Colosseo (F) | 3/10 | 25/134/(19) | 69·3 | 32·0 | 40·0 | 20·0 | 8·0 | 9·2 | MS |
| Creso (G + H) | 0/7 | 0/17/(0) | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 | HR |
| Duilio (A) | 1/9 | 4/129/(3) | 25·0 | 25·0 | 25·0 | 25·0 | 25·0 | 1·8 | R |
| Lloyd (F + H) | 3/20 | 9/138/(6) | 36·2 | 22·2 | 0·0 | 0·0 | 77·8 | 5·1 | MS |
| Mexa (D) | 3/4 | 18/19/(95) | 100·0 | 0·0 | 0·0 | 5·5 | 94·5 | 93·4 | HS |
| Neodur (G) | 2/7 | 2/30/(7) | 20·5 | 100·0 | 0·0 | 0·0 | 0·0 | 1·0 | R |
| Simeto (E + H) | 3/7 | 4/27/(15) | 75·0 | 25·0 | 50·0 | 0·0 | 25·0 | 8·3 | MS |
| Tetradur (F + H) | 5/18 | 27/191/(14) | 63·6 | 29·6 | 22·3 | 44·4 | 3·7 | 7·5 | MS |
| Vitron (D) | 1/9 | 3/172/(2) | 16·0 | 0·0 | 33·3 | 0·0 | 66·6 | 1·4 | R |
| WL-711 (D-I)[f] (control) | 21/41 | 138/383/(36) | 72·7 | 26·0 | 26·8 | 29·7 | 17·5 | 21·2 | HS |
| WL-711 (water control) | 0/34 | 0/292/(0) | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 | 0·0 | – |

[a]Where cultivars were tested more than once, the combined data are given.

[b]Letters in brackets indicate cultivars inoculated on the same date and their respective WL-711 control batch.

[c]Artificially vernalized cultivars.

[d]CI = [(0·25 × seeds in Cat. 0·1 to 1) + (0·5 × seeds in Cat. 2) + (0·75 × seeds in Cat. 3) + (1·0 × seeds in Cat. 4)] × 100/total no. of grains (Cat. 0–4).

[e]HR, highly resistant; R, resistant; MS, moderately susceptible; S, susceptible; HS, highly susceptible.

[f]WL-711 controls are the same for the spring and durum wheats.

Table 2 Morphological susceptibility of European winter (A), spring (B) and durum (C) wheat cultivars to *Tilletia indica*: incidence (ears and seeds infected), coefficient of infection (CI) and categorization of susceptibility for each cultivar compared to a highly susceptible Indian cultivar WL-711, following spray inoculation at mid heading (growth stage 55)

| Cultivar | Ears infected/total number | Seeds infected/ total number (% infection) | Mean % seeds infected/ infected ear | CI[c] |
|---|---|---|---|---|
| A – winter wheat cultivars[a] | | | | |
| Centauro (A) | 4/11 | 24/366/(7) | 8·6 | 2·6 |
| Charger (A + B) | 9/24 | 77/880/(9) | 11·8 | 6·5 |
| Drifter (A) | 1/12 | 5/629/(1) | 0·8 | 0·7 |
| Equinox (B) | 2/11 | 5/326/(2) | 1·4 | 0·5 |
| Kosack (B) | 10/12 | 136/441/(31) | 36·5 | 15·8 |
| Kris (B) | 3/12 | 10/396/(3) | 2·5 | 0·8 |
| Malacca (B) | 0/12 | 0/377/(0) | 0·0 | 0·0 |
| Mikon (A) | 6/12 | 68/609/(11) | 13·5 | 7·2 |
| Mjolner (C) | 4/11 | 16/251/(6) | 6·8 | 2·8 |
| Serio (A) | 9/12 | 56/430/(13) | 13·6 | 6·6 |
| WL-711 (B) | 9/12 | 45/199/(23) | 19·6 | 9·7 |
| WL-711 Boot Control GS 45 (A + B + C) | 32/34 | 378/606/(62) | 58·5 | 39·4 |
| B – spring wheat cultivars[b] | | | | |
| Anza (D + E) | 9/19 | 29/110/(26) | 26·9 | 10·4 |
| Bolero (D + E) | 7/17 | 41/198/(21) | 11·5 | 7·6 |
| Chablis (D + E) | 0/9 | 0/46/(0) | 0·0 | 0·0 |
| Imp (D + E) | 0/2 | 0/14/(0) | 0·0 | 0·0 |
| Pandas (D + E) | 6/12 | 10/50/(20) | 23·6 | 6·5 |
| Sagittario (D + E) | 1/8 | 1/56/(2) | 12·5 | 1·8 |
| Saxana (D + E) | 4/13 | 12/141/(8) | 12·6 | 3·9 |
| C – durum wheat cultivars[b] | | | | |
| Anton (D) | 6/12 | 18/48/(37) | 47·2 | 13·0 |
| Colosseo (D + E) | 3/8 | 4/20/(20) | 22·9 | 5·0 |
| Creso (D) | 1/6 | 1/17/(6) | 8·3 | 1·5 |
| Lloyd (D) | 7/9 | 20/56/(36) | 39·6 | 17·0 |
| Mexa (D + E) | 3/3 | 7/8/(87) | 70·0 | 56·2 |
| Simeto (D + E) | 8/15 | 19/46/(41) | 39·3 | 13·6 |
| Tetradur (D + E) | 0/5 | 0/23/(0) | 0·0 | 0·0 |
| Vitron (D + E) | 13/18 | 34/62/(55) | 59·2 | 14·5 |
| WL-711 (D + E)[d] | 16/27 | 73/151/(48) | 44·5 | 21·4 |

[a]Winter cultivars were inoculated in three batches on separate dates, as indicated by the letters A, B, C.
[b]Two separate experiments were done for spring and durum wheats, as indicated by the letters D and E; data was combined where possible.
[c]CI = [(0·25 × seeds in Cat. 0·1 to 1) + (0·5 × seeds in Cat. 2) + (0·75 × seeds in Cat. 3) + (1·0 × seeds in Cat. 4)] × 100/total no. of grains (Cat. 0–4).
[d]WL-711 controls are the same for the spring and durum wheats.

## Growth of plants

### *Winter wheat cultivars*

For physiological susceptibility tests, plants of each European winter wheat cultivar were raised outside at CSL, UK. Seeds (five seeds per pot) were sown directly in 1 L pots containing John Innes No. 3 compost incorporating a slow release fertiliser (Osmocote at a rate of 3 g $L^{-1}$; N18:P11:K10; controlled release over 8–9 months). Plants of the Indian spring wheat cultivar WL-711 were sown in batches at weekly intervals for use as comparative controls. The pots of WL-711 were grown outside alongside the winter cultivars. Batches of European winter cultivars were grown that were either naturally vernalized by growing them through the winter, or artificially vernalized (seeds germinated on moist tissue paper until the coleoptile is just emerging, then seedlings were incubated at 0°C for 8 weeks) prior to planting. Plants were brought into a quarantine controlled-environment room (20°C; 50% RH; 18 h day length under fluorescent light with tungsten lamp supplement) just prior to inoculation and arranged in randomized blocks. Due to differences in growth rates between cultivars, plants of 10 cultivars were from naturally vernalized batches, whilst plants of five cultivars were from artificially vernalized plants. Previous work (Inman *et al*., 2001) had shown that the method of vernalization did not affect disease susceptibility. In the morphological susceptibility experiment (spray inoculation), all winter cultivars were naturally vernalized by growing them outside, as described above. Plants were brought into the quarantine controlled-environment room (17°C; 50% RH; 16 h day length) just prior to inoculation. Pots of each cultivar were arranged in randomized blocks within the growth room. Inoculations had to be spread over several dates due to differences in growth stages between cultivars. Once in the growth room, plants were

fed every 2 weeks with a standard liquid feed applied directly to the compost.

*Spring and durum cultivars*

For European spring and durum cultivars, seeds were placed on moistened blotter paper in plastic trays and incubated for 3 days at room temperature to start germination at CRA-PAV, Italy. The trays were then transferred into a cold room at 4°C for 4 weeks. After this vernalization, seedlings were transplanted (eight per pot) into 1 L plastic pots containing a 3:1 v/v mixture of potting soil ('Rafleur', VeldKamp Veenproducten) and perlite ('Agriperlite', BPB Vic) and moved into a greenhouse (20 ± 5°C, natural lighting). One week after transplanting, the plants were thinned to six plants per pot and the lighting was supplemented with artificial light (12 h/day). Plants were fed every 2 weeks with a standard liquid feed applied directly to the pots. When plants had reached the growth stage for inoculation they were inoculated in a quarantine facility and then transferred to quarantine growth chambers with a 12 h light cycle (fluorescent light with tungsten lamp supplement) at 20/15°C (day/night) and 60% RH, where they were arranged in randomized blocks.

## Inoculum production

The inoculum was prepared according to Bonde *et al.* (1996) using a mixed population of teliospores from three isolates (Blythe, USA, 1995; Ropar, India, 1997; Sonora, Mexico, 1996). Sporidial inoculum (allantoid secondary sporidia) was prepared from mycelial plugs stored at –80°C, cultured onto potato dextrose agar (PDA) for 6–7 days, transferring the resulting mycelium onto tap water agar (TWA) and then incubating at 20°C with a 12 h white light cycle for 3–4 weeks to obtain sporidia. Inoculum was prepared from 3–5 TWA plates of each of the three *T. indica* isolates to produce a mixed inoculum. For every plate, 2–3 mL of distilled water were added and the agar surface scraped with a disposable spreader. If allantoid sporidia were seen on the inside of the Petri dish lids, 1–2 mL of distilled water was added to the lid to also recover these. The collected suspension was filtered through a 100 $\mu$m screen and adjusted to $1 \times 10^4$ allantoid sporidia $mL^{-1}$ for boot injection method (physiological susceptibility) or to $5 \times 10^4$ for the spray-inoculation method (morphological susceptibility). The suspensions were put on ice and used immediately for inoculation.

## Plant inoculation

*Physiological susceptibility*

For each European cultivar, one head in each of 10 pots was inoculated by injecting 1 mL of spore suspension ($1 \times 10^4$ allantoid sporidia $mL^{-1}$) into the boot cavity (at GS 45) and one head per pot was inoculated by injecting 1 mL of water, as a negative control, to give a total of 10 replicates per treatment. Due to differences in the rate of development, the cultivars were inoculated at different dates depending on the time they reached the boot swollen stage. At each inoculation date, 10 plants of WL-711 were also inoculated at GS 45 by injecting 1 mL of spore suspension to allow comparison between the batches of inoculum used, and 10 plants were treated in the same way with distilled water. Plants of each pot, including controls, were covered with transparent polyethylene bags to maintain a high humidity for infection. The bags were removed after 3 days.

On each inoculation date, the quality of the inoculum was also assessed *in vitro*. Six drops (*ca.* 0·2 mL) of the inoculum were placed onto each of three 3-day-old TWA plates and spread with a sterile spreader. The plates were incubated at 20°C and twenty randomly encountered allantoid sporidia per plate were assessed for germination after 0 and 16–24 h. The inoculum used on all dates was prepared from the same batch of TWA plates.

*Morphological susceptibility*

The optimum time (GS 55, ear half emerged) for spray inoculation was chosen on the basis of previous studies (Singh & Krishna, 1982; Nagarajan *et al.*, 1997; Kumar & Nagarajan, 1998) on growth stage susceptibility, including those done within the Karnal bunt risks EU research project by Bioforsk, Norway (Magnus *et al.*, 2004). One head in each of 10 pots of each wheat cultivar was inoculated by spraying *ca.* 1 mL of spore suspension ($5 \times 10^4$ allantoid sporidia $mL^{-1}$) on to all exposed surfaces of each ear (and flag leaf sheath) as a fine mist. A second head per pot was sprayed with water as a negative control. There were therefore 10 replicates per treatment. At each inoculation date, 10 plants of WL-711 were also spray inoculated with the sporidial inoculum as positive/comparative controls; WL-711 water controls were also included. In addition, at CSL, plants of WL-711 were boot injected (GS 45) to allow comparison between the batches of inoculum used. Small sealable bags were placed over each individual ear and then a transparent polyethylene bag over the whole plant/pot, as described before, to maintain high humidity for infection; all bags were removed after 3 days. On each inoculation date, the viability of the inoculum was also assessed *in vitro*, as described above for the boot injection experiments.

## Plant protection

For durum and spring wheat cultivars at CRA-PAV, a liquid treatment with the fungicide benomyl (0·3 g $L^{-1}$) was applied to pots by spraying the plants and the soil surface immediately after transplanting. This was to prevent attacks from *Fusarium* spp., sporadically observed on germinating seeds of some cultivars. To control powdery mildew (*Blumeria graminis*), the fungicides propiconazole (systemic) and/or fenarimol (locally systemic) were used as necessary at a rate suggested from the commercial products; however, propiconazole was not used within the 4-week period prior to inoculation with *T. indica* to avoid interference with disease development; only foliar sprays of fenarimol were applied, if required. To control aphids

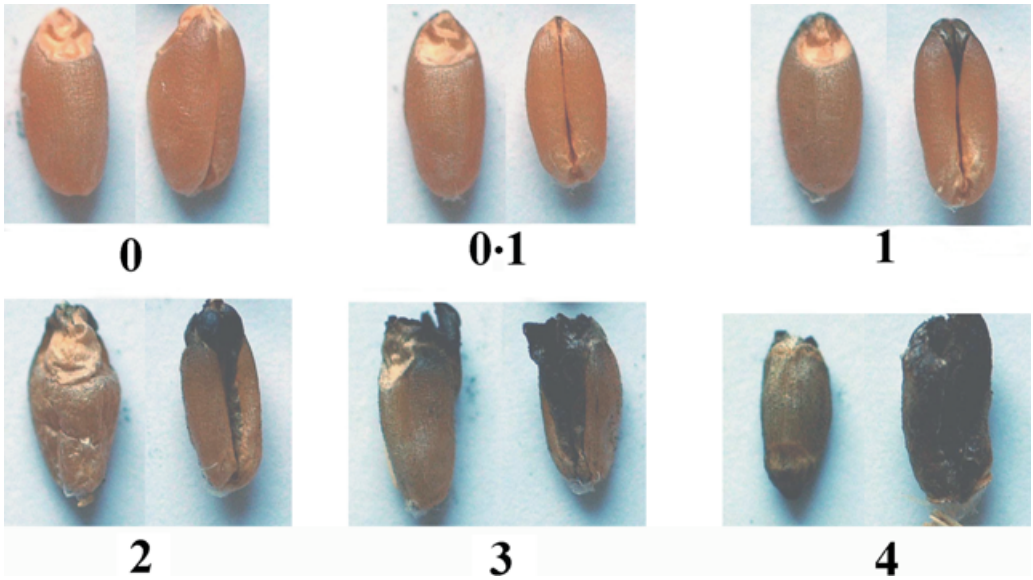


Figure 1 Rating scale used to assess the severity of symptoms of Karnal bunt, based on Aujla *et al.* (1989) and Bonde *et al.* (1996), modified by adding Category 0·1: 0, healthy; 0·1, inconspicuous point infection (*c.* 5% seed bunted); 1, well developed point infection (*c.* 25% seed bunted); 2, infection spreading along the groove (*c.* 50% seed bunted); 3, three-quarters of seed converted to sorus (*c.* 75% seed bunted); 4, seed completely converted to sorus (*c.* 100% seed bunted).

in the quarantine growth room, plants of winter wheat cultivars were sprayed with nicotine in the physiological susceptibility experiment and deltamethrin in the morphological susceptibility experiment, while spring and durum plants were treated with pirimicarb, when needed, at the label rate. Plants of the control cultivar WL-711 were treated in the same way.

### Disease assessment

The ears were collected at maturity (GS 92) by cutting the stem immediately below the ear. The seeds were carefully removed from each ear in a quarantine Class 2 safety cabinet within quarantine facilities. The data collected included the numbers of infected and uninfected seeds per ear to evaluate the incidence of the disease (% seeds infected), and the degree of infection, to evaluate the severity of the disease. This was based on a six point rating scale: 0, healthy seed; 0·1, inconspicuous point infection at the germinal tip (trace); 1, well developed point infection at the germinal tip; 2, infection spreading along the groove; 3, three-quarters of seed converted to sorus; and 4, seed completely converted to sorus (Fig. 1). This scale is based on those used by Aujla *et al.* (1989) and Bonde *et al.* (1996), modified by including an additional infection category (0·1). This additional category was recombined with Category 1 for the subsequent analyses for comparison with published data. Each seed was visually assessed and, where necessary for point infections, observed under a magnifying lens or by microscopy to evaluate the blackening (sorus) and the presence of teliospores. Numerical values of 0, 0·25, 0·50, 0·75 and 1 were assigned to the infection categories 0, 1, 2, 3 and 4, respectively; values were multiplied by the number of seeds assigned in each category, a gross total was obtained and a coefficient of infection was calculated (CI = gross total × 100/total no. of seeds). The CI obtained was used to assign each cultivar tested by boot injection to a 'susceptibility category' as follows: for CI equal to 0 (highly resistant; HR); from 0·1 to 5 (resistant; R); from 5·1 to 10 (moderately susceptible; MS); from 10·1 to 20 (susceptible; S); above 20·1 (highly susceptible; HS) (Aujla *et al.*, 1989).

## Results

At both CSL and CRA-PAV, most of the pot-grown cultivars, raised under the conditions outlined above, produced sufficient ears for inoculation. Only a few cultivars produced few ears, e.g. the spring cultivars Axona, Colfiorito and Quattro produced 4–6 ears (Table 1B), and the durum cultivars Betadur and Mexa produced only one and four ears, respectively (Table 1C). In these cases, results should be interpreted with caution.

### Physiological susceptibility

The physiological susceptibility of European wheat cultivars to *T. indica* by boot injection is summarised in Table 1.

*Winter wheat cultivars*

All cultivars except Kris and Malacca developed Karnal bunt symptoms after boot injection. For the other thirteen European winter wheat cultivars, the total seeds infected ranged from 1 to 32%, compared with 50% for the highly

susceptible Indian spring cultivar (WL-711). The two Italian cultivars Serio and Centauro showed the highest incidence of infection with 28 and 32% of seeds infected, respectively, followed by the two Scandinavian cultivars Kosack and Mjølner with 21 and 22% of seeds infected, respectively. The proportion of seeds in each infection category generally decreased with increasing severity. However, there were differences in the severity of infection between cultivars (Table 1A): the four winter cultivars Aztec, Claire, Equinox and Isengrain exhibited only point infections; the three cultivars Centauro (Italian), Kosack (Scandinavian) and Mikon (Polish), as well as the control cultivar WL-711, exhibited the most severe infections, with some seeds (6, 12, 18 and 10%, respectively) appearing completely bunted (infection category 4).

All the European cultivars were categorized as less susceptible than the highly susceptible Indian cultivar WL-711, based on their CI. Two European cultivars (Centauro and Kosack) were categorized as susceptible; two cultivars (Serio and Mjølner) as moderately susceptible; nine cultivars (Aztec, Charger, Claire, Drifter, Equinox, Flair, Isengrain, Mikon, Soisson) as resistant; and two cultivars (Kris and Malacca) as highly resistant.

*Spring wheat cultivars*

Only two cultivars (Axona and Quattro) did not develop Karnal bunt symptoms after boot injection. For the other thirteen spring wheat cultivars, the incidence of seeds infected ranged from 1 to 48%, compared with 36% for the highly susceptible Indian spring cultivar (WL-711). In general, the severity of infection was equivalent to that observed on the winter cultivars and lower than on the susceptible Indian spring cultivar WL-711. Similarly, spring cultivars also mostly exhibited only point infections (Table 1B), whereas the highly susceptible control cultivar (WL-711) showed a similar proportion of seeds in each of the four infection categories. However, four spring cultivars had a large proportion of seeds completely converted to sori: Colfiorito (100%), Thasos (42%), Imp (33%) and Chablis (30%). All the European spring cultivars were categorized as less susceptible than the highly susceptible Indian cultivar WL-711, based on their CI. Only the Spanish spring cultivar Anza was categorized as susceptible; five cultivars, one English (Chablis), one German (Thasos) and three Italian (Colfiorito, Pandas and Sagittario) were categorized as moderately susceptible; and seven cultivars (Bolero, Cartaya, Imp, Naxos, Sandra, Saxana and Cezanne) as resistant. The UK cultivar Axona and the German cultivar Quattro were not infected and were categorized as highly resistant, but these results could not be considered completely reliable because of the lower number of plants inoculated (Table 1B).

*Durum wheat cultivars*

Only two of the eleven durum cultivars (Creso and Betadur) did not develop Karnal bunt symptoms after boot injection. However, the results for these two cultivars were not considered completely reliable because of the poor quantity of seeds produced in this trial. For the other nine durum cultivars (Table 1C), the total seeds infected was very variable, ranging from 2% for the French cultivar Vitron to 95% for the Spanish cultivar Mexa. Three other durum cultivars showed a higher incidence of infection, the Italian cultivars Colosseo (19%) and Simeto (15%), and the French cultivar Tetradur (14%). In contrast to the winter and spring cultivars, the severity of infection was high: in all but one cultivar, some seeds appeared completely converted to sori (infection category 4); across all infected durum cultivars 34% of seeds fell into category 4, compared to 17% for the highly susceptible Indian spring cultivar (WL-711). Only the cultivar Neodur exhibited only point infections. Although the severity of seed infection was generally high, only the Spanish cultivar Mexa was categorized as highly susceptible, as well as the Indian cultivar WL-711. Because of the relatively low number of seeds infected, the cultivars Simeto, Colosseo, Lloyd and Tetradur were categorized as moderately susceptible, and the cultivar Anton, Duilio, Neodur and Vitron were categorized as resistant.

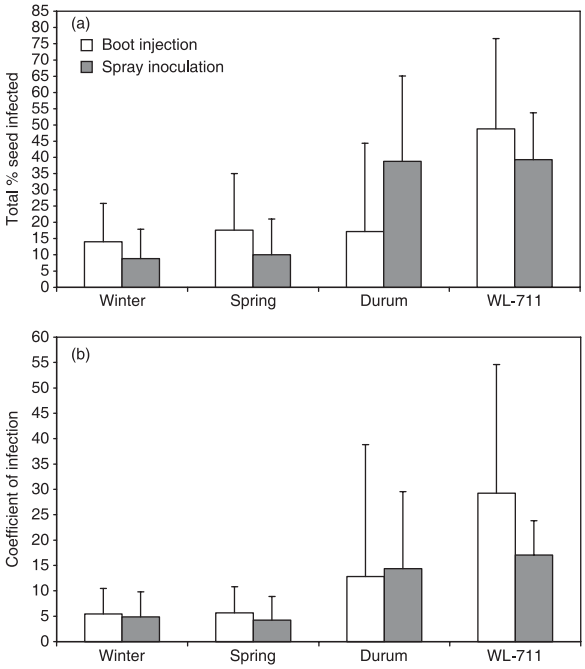


Figure 2 Comparison of the averages of the total percentage seeds infected (a) and the coefficient of infection (b) for 10 winter, seven spring and eight durum European wheat cultivars and the Indian spring cultivar WL-711 inoculated by injection with allantoid sporidia of *Tilletia indica* at growth stage 45 (boot swollen) or spray inoculated at growth stage 55 (ear half emerged). Error bars indicate standard deviation of the mean.

## Morphological susceptibility

The morphological susceptibility of European wheat cultivars to *T. indica* is summarised in Table 2. Figure 2a compares the different wheat types (WL-711 and European winter, spring, durum cultivars) based on their total

percentage of infected seeds following spray inoculation (morphological susceptibility) at GS 55, and the same cultivars by boot injection (physiological susceptibility) at GS 45; Fig. 2b compares their coefficient of infection.

*Winter wheat cultivars*

All cultivars except Malacca developed Karnal bunt symptoms after spray-inoculation (Table 2A). The total seeds infected ranged from 1 to 31% compared to 23% for the comparative Indian control (WL-711). Some cultivars showed levels of infection as high as the comparative Indian control (e.g. Kosack, Serio, Mikon, Charger), whilst others showed much lower levels. The morphological susceptibility of individual winter cultivars broadly reflected their physiological susceptibility. The most physiologically susceptible cultivars, WL-711, Serio and Kosack, also showed significant morphological susceptibility (23, 13 and 31% seeds infected, and 9·7, 6·6 and 15·8 of CI, respectively); however, Mjølner and Centauro showed lower morphological susceptibility than expected (6 and 7% seeds infected, and 2·8 and 2·6 of CI, respectively). For cultivars which were more physiologically resistant, Drifter and Equinox showed comparable morphological susceptibility; however, Charger and Mikon showed greater than expected morphological susceptibility. No infection occurred on Malacca, consistent with its highly resistant rating based upon boot injection (physiological susceptibility).

*Spring and durum cultivars*

In the first experiment with both spring and durum cultivars, poor seeds were produced in some cultivars, probably due to the experimental conditions. The experiments were repeated, but in the repeat experiment, the durum cultivars, Mexa and Tetradur were sterile. The results in Table 2B/C report the combined data from the original and repeated experiments where appropriate. The total seeds infected ranged from 2 to 26% for spring cultivars and 6 to 87% for durum cultivars; this compared with 48% for the Indian control cultivar (WL-711).

The results from both boot injection and spray inoculation experiments showed that the morphological susceptibility of individual spring and durum cultivars tended to reflect their physiological susceptibility. The spring cultivars Anza, Bolero, Imp, Pandas and Saxana, and the durum cultivars Colosseo, Lloyd, Mexa and Simeto were all morphologically susceptible, confirming the earlier boot injection tests that showed they were physiologically susceptible. Mexa was still the most susceptible durum cultivar (87% of seeds infected); it was the only cultivar that was more susceptible than the Indian control cultivar WL-711 based on its CI. However, there were some differences between relative morphological and physiological susceptibility: the durum cultivars Anton and Vitron showed significant morphological susceptibility (37 and 55% of seeds infected, and 13·0 and 14·5 of CI, respectively) despite having relatively low susceptibilities determined by boot inoculation (7·8 and 1·7% of seeds infected, and 4·4 and 1·4 of CI, respectively); in contrast, the spring cultivar Chablis showed a very high morphological resistance (0% of seeds infected) despite being physiologically categorized as moderately susceptible. The only cultivars that were not infected in both spray-inoculation experiments were the spring cultivars Chablis and Imp; the latter also had a very low CI by boot inoculation (0·8) and was categorized as resistant. Although the durum cultivar Creso always produced only a few seeds, only one infected seed (6%) was produced in the first morphological susceptibility experiment, suggesting some resistance to infection.

## Discussion

Until recently, Karnal bunt has been a disease restricted to countries which grow autumn-sown, spring cultivars of *T. aestivum* or *T. durum* under irrigation, e.g. India, Pakistan, Mexico. In these countries, cultivars show a diversity of susceptibility, ranging from highly susceptible to various degrees of resistance. The proportion of resistant lines is higher in triticale, followed by durum wheat, while no bread wheat is completely immune (Singh & Dhaliwal, 1989; Rajaram & Fuentes-Davila, 1998). In general, *T. durum* cultivars are considered less susceptible than *T. aestivum*. Bonde *et al*. (1996) reported that even for the most resistant wheat cultivar tested by them, the percentage of seed infected ranged from 10 to 30% according to the aggressiveness of the isolates used. During these experiments (Bonde *et al*., 1996), no evidence for the existence of races among the field population of the pathogen was detected.

Host resistance has an important role in the management of Karnal bunt because the pathogen is difficult to control chemically. Teliospores resist physical and chemical treatments (Smilanick *et al*., 1985, 1988) and chemical control measures rely mainly on fungicide programmes applied from flag leaf emergence to the end of flowering (Singh *et al*., 1989; Salazar-Huerta *et al*., 1997; Goel *et al*., 2000; Singh *et al*., 2000). Karnal bunt breeding programmes have traditionally screened germplasm for resistance/susceptibility using artificial inoculation methods, and conventionally the injection of allantoid sporidia in the boot cavity is the method used for large-scale screening of germplasm lines.

The original PRA for the UK and the EU (Sansford, 1996, 1998) that was initiated after *T. indica* was introduced into the USA, predicted the risk of entry and establishment based on several key parameters: a suitable trade pathway allowing entry; the presence of susceptible hosts (wheat and triticale); the potential for the pathogen to survive between crops; and the occurrence of the appropriate climatic conditions for infection and disease development. At the time of the earlier PRA, the susceptibility of European wheat cultivars was unknown but they were presumed to be susceptible because there were no breeding programmes for selection for resistance to *T. indica*. It was uncertain as to whether winter cultivars (cultivars that require vernalization) were susceptible to *T. indica* because the disease almost exclusively occurred in areas

where such cultivars had not been grown. It is therefore interesting to note that during the northward spread of Karnal bunt within the state of Texas in 2001/2, *T. indica* was found for the first time on hard red winter wheat in San Saba County (Marshall *et al*., 2003).

FAOSTAT data (FAO, 2007) showed that in 2005 the 25 EU Member States produced *ca*. 124 million tonnes of wheat with France, Germany and the UK being the largest producers (36, 23 and 14 million tonnes, respectively). The main producers of common wheat were France, Germany and the UK, while the main producers of durum wheat were Italy and Spain (data from DAFRD: A. Kinsella, Teagasc, Dublin, personal communication).

The 41 European wheat cultivars tested in this EU project were representative of the main cultivars grown across the EU in 2000/2001. The range of susceptibility was generally similar for winter, spring and durum wheat cultivars. When boot injected, 35/41 cultivars developed Karnal bunt symptoms; when spray inoculated to test morphological (field) susceptibility, 21/25 cultivars developed symptoms. Only the winter wheat cultivar Malacca was not infected using either inoculation method. The range of susceptibility found in the current experiments with European cultivars was similar to that found in cultivars grown in Karnal bunt countries (Singh & Dhaliwal, 1989; Rajaram & Fuentes-Dávila, 1998). There did not appear to be any differences in susceptibility linked to rate of maturity or the end use of the cultivar.

In this current project, the methodology used proved satisfactory in determining susceptibility, although the plant growth conditions showed some limitations, namely: (i) the grain weights of most winter cultivars tested at CSL were small and therefore levels of infection may have been affected; and (ii) the spring and durum cultivars tested at CRA-PAV in the spray-inoculation experiment produced poor seeds in some cases, thereby not allowing an accurate determination of susceptibility. Because *T. indica* is an I/AI quarantine pest for the EU, these experiments had to be done under containment conditions, which are not conducive to normal plant growth.

For winter wheat cultivars, the incidence and severity of infection was generally lower than that found in recent parallel studies on European winter cultivars. Peterson & Creager (2000) reported 9 to 94% seed infection for 52 US and European winter wheat cultivars tested by boot injection ($1 \times 10^4$ allantoid sporidia $mL^{-1}$). Charger and Soisson, the only cultivars common in both studies, showed 10 and 2% seed infection, respectively, in this current study compared to 28 and 49% in Peterson & Creager's study (average of 3 years inoculation; Porta-Puglia *et al*., 2002). Inman *et al*. (2001) found 16 to 94% seed infection for six UK winter cultivars when boot injected with $1–2 \times 10^5$ allantoid sporidia $mL^{-1}$ and all but one cultivar were classified as susceptible or highly susceptible. The lower levels of infection in the current study may have been due to the small size of the grains that developed under the test conditions, which were correlated to reduced development of the sorus. Similar results were obtained by USDA-ARS in India with selected winter wheat cultivars and breeding lines from all regions of the USA. Among the 286 US winter wheat entries tested by boot injection at a nursery at Punjab Agricultural University, Ludhiana, the percentage of seeds infected with Karnal bunt ranged from 2·6 to 100% (Anonymous, 2005).

In conclusion, 35 of 41 European wheat cultivars (85%) were shown to be physiologically susceptible to *T. indica*. This is not unexpected as there has been no targeted breeding for resistance to *T. indica* in European breeding programmes. Some cultivars showed a significant degree of resistance. However, the small number of cultivars that were highly resistant were not the most widely grown. This implies that if *T. indica* was introduced into Europe, any breeding programmes to replace the leading current susceptible cultivars with more resistant cultivars would take some considerable time and would be extremely costly.

Of greatest significance for the EU is the confirmation that under the inoculation protocols in this study, winter cultivars of *T. aestivum* were equally susceptible to infection by *T. indica* as spring cultivars. For *T. aestivum*, there were no obvious differences in susceptibility in relation to end use (bread/biscuit/feed) or maturity types (early/late). Susceptibility of durum wheat cultivars was more variable, although the overall results are rather similar to those obtained with the cultivars of *T. aestivum*. Normally, *T. durum* cultivars are reported as less susceptible than *T. aestivum* cultivars (Singh & Dhaliwal, 1989). This is perhaps of some concern, since European durum wheat production areas, especially in Italy, have been shown to be highly favourable to *T. indica* infection/disease development. This was based on the work done in support of the determination of the risk of establishment by Baker *et al*. (2004) which combined the Humid Thermal Index disease model of Jhorar *et al*. (1992) with bread and durum wheat phenology models, outputting the results into a series of risk maps which were incorporated into the EU PRA (Sansford *et al*., 2006). Also, these areas have a significant pathway of introduction based on interceptions by Italy from Mexico (Inman *et al*., 2008).

Moreover, parallel research on Emmer wheat (*Triticum dicoccum*), a species having an expanding niche-market in some areas of Italy, has shown that this species is also physiologically susceptible to *T. indica* (Riccioni *et al*., 2006).

The results presented here demonstrate that in the presence of disease-conducive environmental conditions, European wheat cultivars are susceptible to *T. indica*. If introduced into Europe, and if other biological requirements, such as teliospore survival and germination in relation to European temperature profiles and wheat crop phenology are met for it to complete its life cycle, then these cultivars could potentially support the establishment of *T. indica* (Peterson *et al*., 2006; Sansford *et al*., 2006; Inman *et al*., 2008). Although a few cultivars showed a significant degree of resistance that could be potentially useful in breeding programmes to develop some resistance to *T. indica* in European wheat cultivars, any such future

breeding programmes are likely to be costly and time consuming (Gill *et al*., 1993).

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